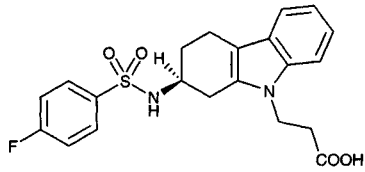
**CHROMATOGRAM**
**Retention time:** 15.168

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

# Ramatroban




**Molecular formula:** $C_{21}H_{21}FN_2O_4S$
**Molecular weight:** 416.48
**CAS Registry No.:** 116649-85-5

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Extract plasma with diethyl ether at pH 6.

**HPLC VARIABLES**
**Guard column:** 20 × 4.6 5 μm Hypersil ODS
**Column:** 125 × 4.6 5 μm Hypersil ODS
**Mobile phase:** MeCN:5mM pH 7.4 tetrabutyl ammonium phosphate buffer 38:62
**Column temperature:** 40
**Flow rate:** 1
**Detector:** UV 284

**CHROMATOGRAM**
**Retention time:** 5

**KEY WORDS**
dog; pharmacokinetics; plasma

**REFERENCE**
Boberg,M.; Ahi,H.-J.; Beckermann,B.; Bühner,K.; Siefert,H.-M.; Steinke,W.; Wünsche,C.; Hirayama,M. Pharmacokinetics and metabolism of the new thromboxane A2 receptor antagonist ramatroban in animals. 1st Communication: Absorption, concentrations in plasma, metabolism, and excretion after single administration to rats and dogs, *Arzneimittelforschung*, **1997**, *47*, 928–938.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Mix 200 μL plasma with IS and extract with diethyl ether at pH 6. Evaporate the ether layer to dryness, reconstitute, inject sample onto column A, elute to waste with mobile phase A, switch the eluate containing ramatroban and IS onto column B between 6.4 and 8.6 min. Backflush the contents of column B onto column C with mobile phase B, elute with mobile phase B, monitor the effluent from column C.

**HPLC VARIABLES**
**Column:** A 250 × 4.6 5 μm Hypersil CPS; B 20 × 4.6 5 μm Hypersil ODS; C 125 × 4.6 5 μm Hypersil ODS
**Mobile phase:** A MeOH:100 mM pH 6 acetate buffer 30:70; B MeOH:THF:pH 6 acetate buffer 33:5:62 (C)
**Column temperature:** 45
**Flow rate:** 1.5
**Detector:** UV 284

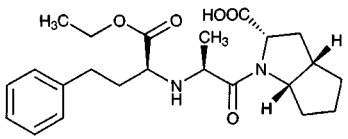
**CHROMATOGRAM**
**Retention time:** 17
**Internal standard:** present but not named
**Limit of quantitation:** 10 ng/mL

**OTHER SUBSTANCES**
**Extracted:** metabolites

**KEY WORDS**
column-switching; heart cut; pharmacokinetics; rat; plasma

**REFERENCE**
Boberg,M.; Ahi,H.-J.; Beckermann,B.; Bühner,K.; Siefert,H.-M.; Steinke,W.; Wünsche,C.; Hirayama,M. Pharmacokinetics and metabolism of the new thromboxane A2 receptor antagonist ramatroban in animals. 1st Communication: Absorption, concentrations in plasma, metabolism, and excretion after single administration to rats and dogs, *Arzneimittelforschung*, **1997**, *47*, 928–938.

# Ramipril



**Molecular formula:** $C_{23}H_{32}N_2O_5$
**Molecular weight:** 416.52
**CAS Registry No.:** 87333-19-5
**Merck Index:** 8283

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

**HPLC VARIABLES**
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 206.4

**CHROMATOGRAM**
**Retention time:** 15.678

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Add MeOH:water 50:50 to powdered capsules or tablets so as to give a ramipril concentration of ca. 40 μg/mL, stir for 15 min, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.5 5 μm Hypersil ODS
**Mobile phase:** MeCN:THF:20 mM pH 2.5 sodium heptanesulfonate 45.6:2.4:52
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 215

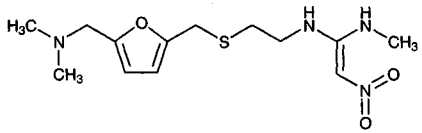
**CHROMATOGRAM**
**Retention time:** 19.0

**OTHER SUBSTANCES**
**Simultaneous:** benazepril

**KEY WORDS**
capsules; tablets

**REFERENCE**
Bonazzi,D.; Gotti,R.; Andrisano,V.; Cavrini,V. Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC), *J.Pharm.Biomed.Anal.*, **1997**, *16*, 431–438.

# Ranitidine



**Molecular formula:** $C_{13}H_{22}N_4O_3S$
**Molecular weight:** 314.41
**CAS Registry No.:** 66357-35-5, 66357-59-3 (HCl)
**Merck Index:** 8286
**Lednicer No.:** 3 131; 4 89, 112, 114

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 100 μL Serum + 100 μL 10 μg/mL IS in water + 50 μL 1 M NaOH, vortex carefully. Add 1 mL dichloromethane and shake for 1 min. Centrifuge at 700 g for 10 min. Add 100 μL 0.1% phosphoric acid to the organic layer, vortex, let stand at room temperature for 5 min. Inject a 50 μL aliquot of the phosphoric acid layer.

**HPLC VARIABLES**
**Column:** 300 × 3.9 10 μm μBondapak C18
**Mobile phase:** MeCN:10 mM pH 3.75 phosphate buffer 15:85
**Flow rate:** 1.0
**Injection volume:** 50
**Detector:** UV 313

**CHROMATOGRAM**
**Retention time:** 4.9
**Internal standard:** AH 20480, N-[3-[5-[[(dimethylamino)methyl]phenoxy]propyl]]-N'-methyl-2-nitro-1,1'-ethenediamine (6.5)
**Limit of detection:** 2 ng/mL
**Limit of quantitation:** 7 ng/mL

**OTHER SUBSTANCES**
**Noninterfering:** amikacin, cefotaxime, metamizole, metronidazole

**KEY WORDS**
serum; pharmacokinetics

**REFERENCE**
Lopez-Calull,C.; Garcia-Capdevila,L.; Arroyo,C.; Bonal,J. Simple and robust high-performance liquid chromatographic method for the determination of ranitidine in microvolumes of human serum, *J.Chromatogr.B*, **1997**, *693*, 228–232.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Condition a 50 mg Bond Elut cyano SPE cartridge (Varian) with 1 mL MeCN and two 1 mL portions of extraction buffer. Add 25 μL N-propionylprocainamide to 500 μL plasma, add 500 μL extraction buffer, vortex for 10s. Add to the SPE cartridge, wash with two 500 μL portions of extraction buffer, elute with 250 μL MeCN:water 50:50, vortex the eluate. Inject a 25 μL aliquot. (The extraction buffer was 100 mM $K_2HPO_4$ adjusted to pH 10.0 with 5 M KOH.)

**HPLC VARIABLES**
**Guard column:** 30 × 4.6 40-50 μm C18 (Alltech)
**Column:** 150 × 3.2 3 μm Hypersil phenyl (Phenomenex)
**Mobile phase:** MeCN:buffer:triethylamine 12:87.9:0.1 (Buffer was 20 mM $K_2HPO_4$ adjusted to pH 6.0 with concentrated phosphoric acid.)
**Flow rate:** 0.6
**Injection volume:** 25
**Detector:** UV 228

**CHROMATOGRAM**
**Retention time:** 5.6
**Internal standard:** N-propionylprocainamide (7.2)
**Limit of detection:** 10 ng/mL

**KEY WORDS**
plasma; SPE

**REFERENCE**
Farthing,D.; Brouwer,K.L.R.; Fakhry,I.; Sica,D. Solid-phase extraction and determination of ranitidine in human plasma by a high-performance liquid chromatographic method utilizing midbore chromatography, *J.Chromatogr.B*, **1997**, *688*, 350–353.

**SAMPLE**
**Matrix:** blood, CSF, tissue
**Sample preparation:** Plasma. 25 μL Plasma + 50 μL 100 μg/mL cimetidine + 100 μL 5 M NaOH + 5 mL dichloromethane, shake for 10 min, centrifuge at 1650 g for 10 min. Evaporate a 4 mL aliquot of the organic phase. Dissolve the residue in 100 μL mobile phase. Inject a 25 μL aliquot. Tissue. Homogenize brain tissue with 100 μL 25 μg/mL cimetidine and 1 mL saline on ice for 1 min. Add 100 μL 1 M NaOH, extract with 5 mL dichloromethane. Evaporate a 3 mL aliquot of the organic phase. Dissolve the residue in 100 μL mobile phase, centrifuge at 10000 g. Inject a 25 μL aliquot. CSF. Inject a 25 μL aliquot of the CSF directly.

**HPLC VARIABLES**
**Column:** 250 × 4 Senshu gel 5C18H (Senshu, Japan)
**Mobile phase:** MeCN:5 mM $NaH_2PO_4$ containing 5 mM tetramethylammonium chloride 5:95
**Column temperature:** 40
**Flow rate:** 2
**Injection volume:** 25
**Detector:** UV 320

**CHROMATOGRAM**
**Internal standard:** cimetidine

**KEY WORDS**
plasma; brain; rat

### REFERENCE

Nakada,Y.; Yamamoto,K.; Kawakami,J.; Sawada,Y.; Iga,T. Effect of renal failure on neurotoxicity of ranitidine in rats, *Biol.Pharm.Bull.*, **1996**, *19*, 323–325.


### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

### HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 228.7

### CHROMATOGRAM

**Retention time:** 3.74

### KEY WORDS

whole blood


### REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.


### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Plasma. 500 μL Plasma + 100 μL 100 mM NaOH, mix, add 3 mL dichloromethane, shake for 10 min, centrifuge at 2000 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of argon, reconstitute with 500 μL mobile phase, inject a 100 μL aliquot. Urine. Dilute 1 mL urine with 25 mL water, filter (0.2 μm), inject a 100 μL aliquot.

### HPLC VARIABLES

**Guard column:** 5 μm Spherisorb ODS-2

**Column:** 150 × 4 5 μm Spherisorb ODS-2

**Mobile phase:** Gradient. MeCN:7.5 mM pH 6 phosphate buffer 7:93 for 8 min, to 25:75 over 1 min, maintain at 25:75 for 6 min, return to initial conditions over 1 min, re-equilibrate for 10 min. (At the end of each day wash column with MeCN then water then re-equilibrate with mobile phase.)

**Flow rate:** 1

**Injection volume:** 100

**Detector:** F ex 350 em 450 following post-column reaction. The column effluent mixed with 5 mM sodium hypochlorite in 50 mM pH 4.5 sodium acetate buffer pumped at 1 mL/min and this mixture flowed through a 0.8 m × 0.5 mm ID PTFE coil at 25°. The effluent from this coil mixed with 20 mM o-phthalaldehyde in EtOH:500 mM pH 10.5 borate buffer 2:98 pumped at 1 mL/min and 100 mM 2-mercaptoethanol in 500 mM pH 10.5 borate buffer pumped at 1 mL/min and this mixture flowed through a 2.5 m × 0.5 mm ID PTFE coil at 25° to the detector.

(The hypochlorite oxidizes the secondary to a primary amine which then reacts with the o-phthalaldehyde and 2-mercaptoethanol.)

**CHROMATOGRAM**
**Retention time:** 13
**Limit of detection:** 32 ng/mL
**Limit of quantitation:** 106 ng/mL

**OTHER SUBSTANCES**
**Extracted:** metabolites

**KEY WORDS**
post-column reaction; plasma

**REFERENCE**
Vinas,P.; Campillo,N.; Lopez-Erroz,C.; Hernandez-Cordoba,M. Use of post-column fluorescence derivatization to develop a liquid chromatographic assay for ranitidine and its metabolites in biological fluids, *J.Chromatogr.B*, **1997**, *693*, 443–449.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Ultrasphere C18
**Mobile phase:** MeOH:THF:20 mM pH 6.0 sodium phosphate buffer 30:67.5:2.5
**Flow rate:** 1
**Detector:** UV 318

**REFERENCE**
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

**SAMPLE**
**Matrix:** solutions

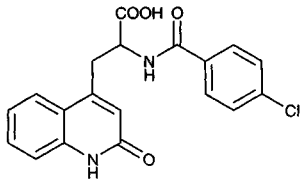
**HPLC VARIABLES**
**Column:** 250 × 4.6 10 μm Partisil ODS1
**Mobile phase:** MeOH:50 mM pH 3.0 phosphoric acid 10:90
**Column temperature:** 30
**Flow rate:** 1.5
**Detector:** radioactivity detection

**OTHER SUBSTANCES**
**Also analyzed:** atenolol, cimetidine, hydrochlorothiazide

**KEY WORDS**
14C labeled

**REFERENCE**
Collett,A.; Sims,E.; Walker,D.; He,Y.-L.; Ayrton,J.; Rowland,M.; Warhurst,G. Comparison of HT29-18-$C_1$ and Caco-2 cell lines as models for studying intestinal paracellular drug absorption, *Pharm.Res.*, **1996**, *13*, 216–221.

# Rebamipide



**Molecular formula:** $C_{19}H_{15}ClN_2O_4$
**Molecular weight:** 370.79
**CAS Registry No.:** 111911-87-6
**Merck Index:** 8296

## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Plasma. 500 μL Plasma + 100 μL 10% metaphosphoric acid + 3 mL toluene, shake for 5 min, centrifuge at 1800 g for 5 min, discard the toluene layer. Add 10 μL 40 μg/mL IS in MeOH, 100 μL 10% metaphosphoric acid, and 500 μL ethyl acetate to the aqueous layer, shake, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 100 μL MeOH, inject a 40 μL aliquot. Urine. 500 μL Urine + 500 μL 500 mM NaOH + 3 mL toluene, shake for 5 min, centrifuge at 1800 g for 5 min, discard the toluene layer. Add 10 μL 100 μg/mL IS in MeOH, 1 mL 10% metaphosphoric acid, and 500 μL ethyl acetate to the aqueous layer, shake, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 200 μL MeOH, inject a 10 μL aliquot.

## HPLC VARIABLES

**Column:** 250 × 4.6 10 μm YMC Pack A-303 ODS (Yamamura)
**Mobile phase:** MeCN:THF:acetic acid:water 32:3:1:64
**Flow rate:** 1.2
**Injection volume:** 10-40
**Detector:** UV 280 or F ex 330 em 375

## CHROMATOGRAM

**Retention time:** 6.2
**Internal standard:** α-(p-chlorobenzamido)-1,2-dihydro-1-methyl-2-oxo-4-quinolinepropionic acid (rebamipide methylated on N of quinoline) (OPC-12 823, Otsuka) (10.0)
**Limit of quantitation:** 500 ng/mL (urine), 10 ng/mL (plasma)

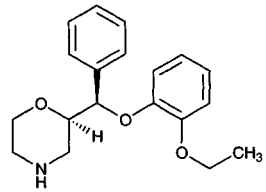
## KEY WORDS

plasma; pharmacokinetics

## REFERENCE

Shioya,Y.; Shimizu,T. High-performance liquid chromatographic procedure for the determination of a new antigastric ulcer agent, 2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propioni c acid, in human plasma and urine, *J.Chromatogr.*, **1988**, *434*, 283–287.

# Reboxetine



**Molecular formula:** $C_{19}H_{23}NO_3$
**Molecular weight:** 313.40
**CAS Registry No.:** 98769-81-4

## SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 1 mL Tris buffer + 7 mL diethyl ether, vortex for 1 min, centrifuge at 1200 g for 5 min. Remove the organic phase and add it to 200 μL 10 mM phosphoric acid, vortex for 1 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 200 μL borate buffer and 200 μL reagent, shake, let stand at room temperature for 5 min, add 100 μL 100 mM L-proline in water, add 3 mL n-hexane, vortex for 1 min. Remove

the organic layer and add it to 1 mL MeCN, extract, discard the upper n-hexane layer, wash the MeCN layer with 1 mL n-hexane. Evaporate the MeCN layer to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject a 200 μL aliquot. (Tris buffer was 25 mL 200 mM Tris solution and 5 mL 100 mM HCl made up to 100 mL with water, pH 9.1. Borate buffer was 61.8 g boric acid in 900 mL water, adjust pH to 8.0 with 20% NaOH, make up to 1 L with water. Prepare reagent by diluting 1 mL 18 mM (+)-1-(9-fluorenyl)ethyl chloroformate in acetone to 50 mL with MeCN.)

**HPLC VARIABLES**
**Guard column:** 30-38 μm Survival pellicular ODS (Whatman)
**Column:** 250 × 4.6 4 μm Supersphere 60 RP-8 (end-capped) (Merck)
**Mobile phase:** THF:buffer 46.5:53.5 (Prepare buffer by dissolving 13.2 g $(NH_4)_2HPO_4$ in 900 mL water, adjust pH to 7.5 with 85% phosphoric acid, make up to 1 L with water.)
**Flow rate:** 0.5
**Injection volume:** 200
**Detector:** F ex 260 em 315

**CHROMATOGRAM**
**Retention time:** 57 (-), 59 (+)
**Limit of quantitation:** 1 ng/mL

**KEY WORDS**
plasma; chiral; pharmacokinetics; derivatization

**REFERENCE**
Frigerio,E.; Pianezzola,E.; Strolin Benedetti,M. Sensitive procedure for the determination of reboxetine enantiomers in human plasma by reversed-phase high-performance liquid chromatography with fluorimetric detection after derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate, *J.Chromatogr.A*, **1994**, *660*, 351–358.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Buffer 1 mL plasma to 9.1 with 50 mM Tris buffer, extract with diethyl ether. Extract the diethyl ether layer with 10 mM phosphoric acid, wash the aqueous layer with n-hexane, inject an aliquot of the aqueous layer.

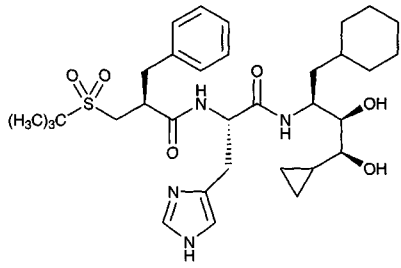
**HPLC VARIABLES**
**Guard column:** CO:PELL:ODS
**Column:** 110 × 4.6 5 μm Partisphere C8 (Whatman)
**Mobile phase:** MeCN:10 mM pH 2.3 phosphate buffer 64:36
**Flow rate:** 0.45
**Detector:** UV 210

**CHROMATOGRAM**
**Internal standard:** phenmetrazine
**Limit of quantitation:** 10 ng/mL

**KEY WORDS**
plasma; pharmacokinetics

**REFERENCE**
Edwards,D.M.F.; Pellizzoni,C.; Breuel,H.P.; Berardi,A.; Castelli,M.G.; Frigerio,E.; Poggesi,I.; Rocchetti,M.; Dubini,A.; Strolin Benedetti,M. Pharmacokinetics of reboxetine in healthy volunteers. Single oral doses, linearity and plasma protein binding, *Biopharm.Drug Dispos.*, **1995**, *16*, 443–460.

# Remikiren



**Molecular formula:** $C_{33}H_{50}N_4O_6S$
**Molecular weight:** 630.85
**CAS Registry No.:** 126222-34-2

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 500 μL Plasma + 5 μL 2 μg/mL IS in MeOH + 1 mL butyl acetate, extract for 2 min, centrifuge at 2000 g for 20 min. Remove the organic layer and evaporate it to dryness under a stream of helium at 60°, reconstitute the residue in 40 μL MeCN and 25 μL 1 M pH 6.3 borate buffer, vortex for 2 min, add 10 μL 25.85 mg/mL 9-fluorenylmethyl chloroformate in MeCN, vortex for 1 min, let stand for 1 h, add 20 μL 225 mM L-proline in water, inject an aliquot.

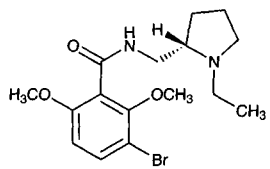
**HPLC VARIABLES**
**Column:** 125 × 4 3 μm Nucleosil C18
**Mobile phase:** A:B 92:8, after 15 min wash with MeCN for 5 min, re-equilibrate at initial conditions for 10 min. A was MeCN containing 350 μg/mL N-hexylmethylamine and 15 mM trifluoroacetic acid, adjusted to pH 3.0. B was 5 mM trifluoroacetic acid in water.
**Flow rate:** 0.75
**Detector:** F ex 261 em 308

**CHROMATOGRAM**
**Retention time:** 8.6
**Internal standard:** (S)-α-[(S)-α-[(tertbutylsulfonyl)methyl]hydrocinnamido]-N-[1S,2R,3S-1-(cyclohexylmethyl)-2,3-dihydroxy-4-methylpentyl]imidazole-4-propionamide (Ro 42-4661) (10.8)
**Limit of quantitation:** 2 ng/mL

**KEY WORDS**
derivatization; plasma; rat; dog; monkey; pharmacokinetics

**REFERENCE**
Coassolo,P.; Fischli,W.; Clozel,J.-P.; Chou,R.C. Pharmacokinetics of remikiren, a potent orally active inhibitor of human renin, in rat, dog and primates, *Xenobiotica*, **1996**, *26*, 333–345.

# Remoxipride



**Molecular formula:** $C_{16}H_{23}BrN_2O_3$
**Molecular weight:** 371.27
**CAS Registry No.:** 80125-14-0, 82935-42-0 (HCl)
**Merck Index:** 8301
**Lednicer No.:** 4 42

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 1 mL Plasma + 100 μL MeCN, mix for 5 s, add 1 mL saturated sodium carbonate, mix for 5 s, add 7 mL pentane:dichloromethane 75:25, shake gently for 10 min, centrifuge at 18° at 1735 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 140 μL MeCN, inject an aliquot.

### HPLC VARIABLES

**Column:** 250 × 4.6 5 μm Ultrasphere cyano
**Mobile phase:** MeCN:MeOH:40 mM pH 6.8 ammonium acetate 82:8:10
**Column temperature:** 40
**Flow rate:** 1.5
**Injection volume:** 150
**Detector:** E, ESA Coulochem model 5100A, model 5011 analytical cell, screening electrode 0.6 V, detection electrode 0.92 V, model 5020 guard cell 1 V

### CHROMATOGRAM

**Retention time:** 17
**Internal standard:** remoxipride
**Limit of detection:** <2.5 ng/mL

### OTHER SUBSTANCES

**Extracted:** risperidone
**Simultaneous:** pseudoephedrine
**Noninterfering:** acetaminophen, benztropine, clonazepam, clozapine, fluphenazine, haloperidol, ibuprofen, lorazepam, trihexyphenidyl

### KEY WORDS

plasma; remoxipride is IS

### REFERENCE

Aravagiri,M.; Marder,S.R.; Van Putten,T.; Midha,K.K. Determination of risperidone in plasma by high-performance liquid chromatograhy with electrochemical detection: application to therapeutic drug monitoring in schizophrenic patients, *J.Pharm.Sci.*, **1993**, *82*, 447–449.

### SAMPLE

**Matrix:** blood, urine
**Sample preparation:** Plasma. 1 mL Plasma + IS in 50 mM pH 2 $NaH_2PO_4$ + 500 μL 1 M NaOH + 4 mL diethyl ether:n-heptane 30:70, rotate for 10 min (if necessary break emulsion by freezing at -20° for 1 h), centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 300 μL pH 2 phosphate buffer, add 750 μL diethyl ether:n-heptane 30:70, vortex for 10 s, inject a 200 μL aliquot of the aqueous phase. Urine. 500 μL Urine + IS in 50 mM pH 2 $NaH_2PO_4$ + 500 μL 0.2 M NaOH + 4 mL diethyl ether:n-heptane 80:20, rotate for 10 min (if necessary break emulsion by freezing at -20° for 1 h), centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 0.5-3 mL pH 2 phosphate buffer, add 750 μL diethyl ether:n-heptane 30:70, vortex for 10 s, inject a 75 μL aliquot of the aqueous phase.

### HPLC VARIABLES

**Column:** 100 × 4.6 3 μm Nucleosil 120-3C18
**Mobile phase:** MeCN:pH 2 phosphate buffer 30:70 containing 0.4 mM N,N-dimethyloctylamine and 0.5 mM decyl sulfate (plasma) or MeCN:pH 2 phosphate buffer 25:75 containing 0.2 mM N,N-dimethyloctylamine (urine)
**Flow rate:** 1.3
**Injection volume:** 75-200
**Detector:** UV 206

### CHROMATOGRAM

**Retention time:** 2.2 (urine), 3 (plasma)
**Internal standard:** 3-bromo-N-[(1-propyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide (3.5 (urine), 5 (plasma))
**Limit of quantitation:** 2 nM

### OTHER SUBSTANCES

**Noninterfering:** metabolites

### KEY WORDS

plasma

**REFERENCE**
Nilsson,L.B. Determination of remoxipride in plasma and urine by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1990**, *526*, 139–150.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** 500 μL Plasma or 50 μL urine + 50 μL 5 μg/mL IS in 100 mM phosphoric acid + 50 μL 100 mM phosphoric acid + 200 μL 1 M NaOH + 5 mL hexane:MTBE 20:80, vortex for 2 min, let stand until the phases separate, freeze in dry ice/acetone. Remove the organic layer and it wash twice with 250 μL portions of 1 M NaOH. Add 500 μL 100 mM phosphoric acid to the organic layer, vortex, let stand for 5 min, discard the organic layer, evaporate any residual organic solvent with a stream of nitrogen, inject a 50 μL aliquot of the aqueous layer.

**HPLC VARIABLES**
**Column:** 50 × 4.6 3 μm Sepralyte C18 (Analytichem)
**Mobile phase:** MeCN:buffer 25:75 (plasma) or 31:69 (urine) (Buffer was 200 mM sodium perchlorate containing 100 mM phosphoric acid, pH 1.7.)
**Column temperature:** 40
**Flow rate:** 1.3
**Injection volume:** 50
**Detector:** UV 214

**CHROMATOGRAM**
**Retention time:** 1.8 (urine), 2.8 (plasma)
**Internal standard:** 3-bromo-N-[(1-propyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide (2.6 (urine), 4.5 (plasma))
**Limit of quantitation:** 50 ng/mL (urine), 12.5 ng/mL (plasma)

**KEY WORDS**
plasma; pharmacokinetics

**REFERENCE**
Chiou,R.H.-Y.; Lo,M.-W. Determination of remoxipride in human plasma and urine by reversed-phase ion-pair high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *581*, 300–305.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 100 × 4.6 7 μm Hypercarb porous graphitic carbon (Shandon)
**Mobile phase:** MeCN:0.1% trifluoroacetic acid 50:50
**Flow rate:** 1
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 3

**REFERENCE**
Gu,G.; Lim,C.K. Separation of anionic and cationic compounds of biomedical interest by high-performance liquid chromatography on porous graphitic carbon, *J.Chromatogr.*, **1990**, *515*, 183–192.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
**Flow rate:** 0.6

**Injection volume:** 25
**Detector:** UV 229

**CHROMATOGRAM**
**Retention time:** 8.84 (A), 4.64 (B)

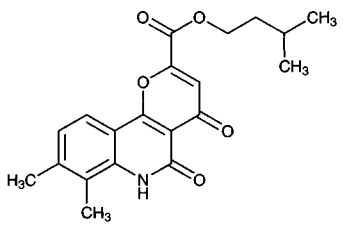
**OTHER SUBSTANCES**
**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

**KEY WORDS**
details of plasma extraction

**REFERENCE**
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

# Repirinast

**Molecular formula:** $C_{20}H_{21}NO_5$
**Molecular weight:** 355.39
**CAS Registry No.:** 73080-51-0
**Merck Index:** 8305
**Lednicer No.:** 5 175



**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Plasma. Adjust pH of 1 mL plasma to 3-4 with 100 µL 1 M HCl, add 1 mL 1 M HCl, add 50 µL 10 µg/mL IS in water, add 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat the extraction. Combine the organic phases and add them to 2 mL 100 mM pH 7.5 phosphate buffer, extract. Remove the aqueous layer and add it to 100 µL PIC A low

UV reagent (tetrabutylammonium bromide, Waters), add the mixture to a Bond Elut C8 SPE cartridge, elute with 7 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 500 μL mobile phase, inject an aliquot. Urine. Dilute 100 μL urine to 10 mL with pH 2 citrate/HCl buffer, inject an aliquot.

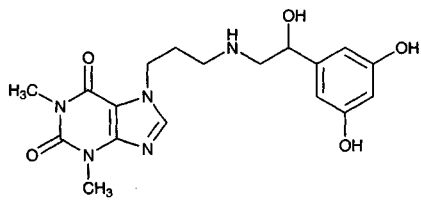
**HPLC VARIABLES**
**Column:** 250 × 4 5 μm Hypersil ODS
**Mobile phase:** MeCN:MeOH:buffer 5:30:65 to 5:45:50 (Buffer was 5 mM pH 3 tetrabutylammonium bromide.)
**Column temperature:** 65
**Flow rate:** 1.2
**Detector:** UV 345

**CHROMATOGRAM**
**Retention time:** 9.3 (as the active metabolite, the free acid, BAY w 8199)
**Internal standard:** BAY × 1453 (10.1)
**Limit of quantitation:** 10 ng/mL

**KEY WORDS**
plasma; pharmacokinetics; SPE

**REFERENCE**
Beermann,D.; Schaefer,H.G.; Wargenau,M.; Heibel,B.; Sturm,Y.; Kuhlmann,J. Pharmacokinetics of the active metabolite of the prodrug repirinast in healthy Caucasian volunteers after a single oral dose, *Eur.J.Clin.Pharmacol.*, **1992**, *42*, 307–312.

# Reproterol




**Molecular formula:** $C_{18}H_{23}N_5O_5$
**Molecular weight:** 389.41
**CAS Registry No.:** 54063-54-6, 13055-82-8 (HCl)
**Merck Index:** 8307

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Condition a 1 mL C18 Bond Elut SPE cartridge with 1mL MeCN and 1 mL 100 mM pH 6.5 ammonium acetate buffer. Mix 250 μL plasma with 10 ng IS, 100 μL 10 mM HCl, and 500 μL 100 mM pH 6.5 ammonium acetate buffer. Make up to 1 mL with 100 mM pH 6.5 ammonium acetate buffer, vortex briefly. Add the mixture to the SPE cartridge. Wash with 400 μL MeCN:2.5 mM pH 6.5 ammonium acetate buffer 20:80, elute with 400 μL mobile phase (also defined as MeCN:2.5 mM pH 6.5 ammonium acetate buffer 80:20). Use the automatic sample processor (ASPEC XL, Gilson, France) to transfer a 100 μL aliquot into autosampler vial. Inject a 100 μL aliquot.

**HPLC VARIABLES**
**Column:** 30 × 4.6 5 μm. Guard column ODS (30) Phenomenex-Ultracarb (Phenomenex, USA)
**Mobile phase:** MeCN:2.5 mM pH 6.5 aqueous ammonium acetate 20:80
**Flow rate:** 1.0
**Injection volume:** 100
**Detector:** MS, Finnigan MAT TSQ 7000 triple-stage-quadrupole, positive mode, collision gas xenon, 133.32 Pa, 20 eV, heated vaporiser temperature 475°, sheath gas nitrogen 344.74 KPa, auxiliary gas nitrogen, exact flow rate not measured, 5 μA, heated capillary temperature 180°., m/z 390

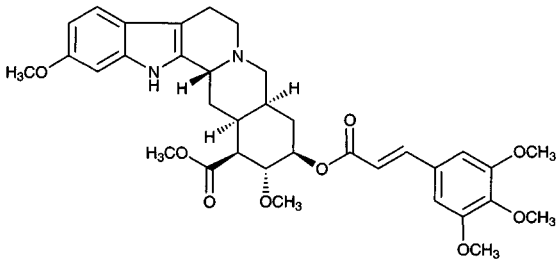
**CHROMATOGRAM**
**Retention time:** 0.3-0.5
**Internal standard:** D-4908 (7-{3-[2-(2,5-dihydroxyphenyl)-2-hydroxyethylamino]-(1-methylpropyl)}theophylline; ASTA Medica, Germany; m/z 404) (0.3-0.5)

**Limit of quantitation:** 400 pg/mL

**KEY WORDS**
plasma; SPE; pharmacokinetics

**REFERENCE**
Knebel,N.G.; Winkler,M. Rapid and automated determination of the β2-agonist reproterol in human plasma by atmospheric pressure chemical ionisation high-performance liquid chromatography--tandem mass spectrometry, *J.Chromatogr.B*, **1997**, *702*, 119–129.

# Rescinnamine



**Molecular formula:** $C_{35}H_{42}N_2O_9$
**Molecular weight:** 634.73
**CAS Registry No.:** 24815-24-5
**Merck Index:** 8311
**Lednicer No.:** 1 319

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 125 × 4.9 Spherisorb S5W silica
**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
**Flow rate:** 2
**Injection volume:** 20
**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

**CHROMATOGRAM**
**Retention time:** 1.4

**OTHER SUBSTANCES**
**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaver-

ine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

## REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

**Column temperature:** 30

**Flow rate:** 2

**Detector:** UV 210

## OTHER SUBSTANCES

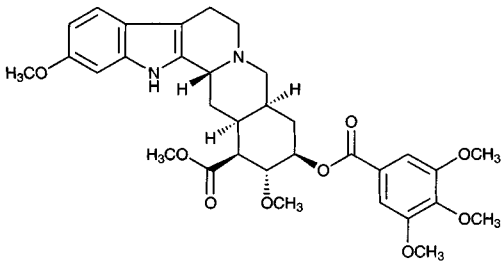
**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendi-

metrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

**REFERENCE**
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

# Reserpine

**Molecular formula:** $C_{33}H_{40}N_2O_9$
**Molecular weight:** 608.69
**CAS Registry No.:** 50-55-5
**Merck Index:** 8314




**SAMPLE**
**Matrix:** blood
**Sample preparation:** 2 mL Plasma + 2 mL saturated sodium borate in water + 3 mL benzene (Caution! Benzene is a carcinogen!), rotate at slow speed for 5 min, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 30 µL reagent, mix well, let stand for at least 10 min, inject a 20 µL aliquot. (Prepare reagent by adding 1 mL saturated vanadium pentoxide in concentrated phosphoric acid to 9 mL MeOH.)

**HPLC VARIABLES**
**Column:** 300 × 4 µBondapak C18
**Mobile phase:** MeOH:10 mM sodium heptanesulfonate 65:35
**Flow rate:** 2.5
**Injection volume:** 20
**Detector:** F ex 390 em 470 (cut-off filter)

**CHROMATOGRAM**
**Retention time:** 6
**Limit of detection:** 100 pg/mL

**OTHER SUBSTANCES**
**Interfering:** rescinnamine

**KEY WORDS**
plasma; horse; comparison with TLC method

**REFERENCE**
Sams,R. Determination of reserpine in plasma using high-performance liquid chromatography with fluorescence detection, *Anal.Lett.*, **1978**, *B11*, 697–707.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 3 mL Plasma + 45 μL 100 ng/mL IS in 10 mM HCl + 1 mL 600 mM pH 9.5 carbonate buffer + 10 mL n-heptane:isoamyl alcohol 98.5:1.5, shake for 10 min, centrifuge for 10 min. remove the organic layer and add it to 1.2 mL 100 mM HCl, shake for 10 min, centrifuge for 10 min. Remove the aqueous layer and add it to 500 μL 600 mM pH 9.5 carbonate buffer, add 500 μL MTBE, mix, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, add 40 μL reagent, let stand for at least 10 min, inject the whole amount. (Reagent was a 1 in 10 mixture of a saturated solution of vanadium pentoxide in MeOH.)

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm LC-1 trimethylsilyl (Supelco)
**Mobile phase:** MeCN:100 mM pH 4.2 acetate buffer containing 5 mM heptanesulfonate and 10 mM triethylamine
**Flow rate:** 1.6
**Injection volume:** 40
**Detector:** F ex 390 em 480

**CHROMATOGRAM**
**Retention time:** 5.2
**Internal standard:** methyl-18-triethoxybenzoylreserpate (7.8)
**Limit of detection:** 0.1 ng/mL
**Limit of quantitation:** 0.3 ng/mL

**OTHER SUBSTANCES**
**Noninterfering:** metabolites

**KEY WORDS**
derivatization; plasma; pharmacokinetics

**REFERENCE**
Suckow,R.F.; Cooper,T.B.; Asnis,G.M. An improved method for the determination of reserpine in plasma using liquid chromatography with fluorescence detection, *J.Liq.Chromatogr.*, **1983**, *6*, 1111–1122.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 3 mL Plasma + 3 mL saturated sodium borate in water + 4.5 mL n-hexane:dichloromethane 50:50, mix for 20 min on a rotating tumbler, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 50 μL reagent, let stand for 25 min, inject a 20 μL aliquot. (Reagent was 1 mL of a saturated solution of vanadium pentoxide in concentrated phosphoric acid and 9 mL MeOH.)

**HPLC VARIABLES**
**Column:** 300 × 3.9 μBondapak C18
**Mobile phase:** MeOH:10 mM sodium heptanesulfonate in water 65:35
**Flow rate:** 2
**Injection volume:** 20
**Detector:** F ex 390 em 470

**CHROMATOGRAM**
**Retention time:** 6.5
**Limit of detection:** 70 pg/mL

**KEY WORDS**
plasma; horse; pharmacokinetics; derivatization

**REFERENCE**
Chapman,C.B.; Courage,P.; Huntington,P.J. Detection of reserpine in horses by high-performance liquid chromatography, *Aust.Vet.J.*, **1991**, *68*, 296–298.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

## HPLC VARIABLES

**Column:** 300 × 3.9 4 µm NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) $KH_2PO_4$ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 268

## CHROMATOGRAM

**Retention time:** 5.94

**Limit of detection:** <120 ng/mL

## KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

**HPLC VARIABLES**
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 218.1

**CHROMATOGRAM**
**Retention time:** 16.433

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

**SAMPLE**
**Matrix:** bulk
**Sample preparation:** Dissolve 25 mg reserpine in 500 μL chloroform, make up to 50 mL with MeOH. Dilute a 20 mL aliquot to 100 mL with MeOH, inject an aliquot.

**HPLC VARIABLES**
**Column:** 300 × 3.9 μPorasil
**Mobile phase:** MeOH or MeOH:20 mg/mL sodium 1-pentanesulfonate in water 100:0.05
**Flow rate:** 0.7
**Injection volume:** 20
**Detector:** F ex 280 em 360

**CHROMATOGRAM**
**Retention time:** 12

**OTHER SUBSTANCES**
**Simultaneous:** ajmalicine

**KEY WORDS**
normal phase

**REFERENCE**
Cieri,U.R. Determination of ajmalicine in reserpine raw materials by liquid chromatography with fluorescence detection, *J.AOAC Int.*, **1995**, *78*, 944–945.

**SAMPLE**
**Matrix:** cell cultures

**Sample preparation:** Extract 5 g cell culture with 20 mL MeOH with sonication for 10 min, repeat extraction twice. Evaporate extracts to dryness under reduced pressure, reconstitute in 100 mL 10 mM HCl, filter, adjust pH to 6 with 10 mM NaOH, inject a 5-100 μL aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4 10 μm Armsorb-300-C8 (Armchrom, Yerevan, Armenia)
**Mobile phase:** Gradient. A was MeCN:water 10:90 containing 0.1% trifluoroacetic acid. B was 0.1% trifluoroacetic acid in MeCN. A:B from 100:0 to 50:50 over 50 min.
**Flow rate:** 0.8
**Injection volume:** 5-100
**Detector:** UV 280

**CHROMATOGRAM**
**Retention time:** 41

**OTHER SUBSTANCES**
**Extracted:** ajmaline, ajmalicine, raucaffricine, serpentine, yohimbine

**REFERENCE**
Klyushnichenko,V.E.; Yakimov,S.A.; Tuzova,T.P.; Syagailo,Y.V.; Kuzovkina,I.N.; Wulfson,A.N.; Miroshnikov,A.I. Determination of indole alkaloids from *R. serpentina* and *R. vomitoria* by high-performance liquid chromatography and high-performance thin-layer chromatography, *J.Chromatogr.A*, **1995**, *704*, 357–362.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Add one crushed tablet or 100 mg Rauwolfia serpentina powder to 6 mL MeOH, swirl to disperse, add 60 mL 250 mM sulfuric acid, mix well, extract five times with 30 mL portions of chloroform, pass extracts through column, collect eluates in 50 mL MeOH, evaporate to 25 mL on a steam bath with an air current (in a hood), add 25 mL MeOH, evaporate to 25 mL, make up to 50 mL with MeOH, mix, inject a 100 μL aliquot. (Column was 3 g Celite 545 and 2 mL 100 mM NaOH in a 20 mm dia glass column.)

**HPLC VARIABLES**
**Column:** 300 × 3.9 μPorasil
**Mobile phase:** MeOH
**Flow rate:** 1.5
**Injection volume:** 100
**Detector:** F ex 280 em 360

**CHROMATOGRAM**
**Retention time:** 5

**OTHER SUBSTANCES**
**Simultaneous:** rescinnamine (F 330 em 435)

**KEY WORDS**
normal phase; reserpine and rescinnamine have same retention time but are discriminated by different detector settings; tablets; powder; rauwolfia serpentina

**REFERENCE**
Cieri,U.R. Determination of reserpine and rescinnamine in *Rauwolfia serpentina* preparations by liquid chromatography with fluorescence detection, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 540–546.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Crush tablets, weigh out amount equivalent to one tablet, add 40 mL MeOH, warm on a steam bath for 5 min, cool to room temperature, make up to 100 mL with MeOH, filter through paper, inject 100 μL aliquot.

**HPLC VARIABLES**
**Column:** 300 × 3.9 μPorasil

**Mobile phase:** MeOH
**Flow rate:** 1.5
**Injection volume:** 100
**Detector:** F ex 280 em 360

**CHROMATOGRAM**
**Retention time:** 4

**OTHER SUBSTANCES**
**Simultaneous:** hydrochlorothiazide

**KEY WORDS**
tablets; normal phase

**REFERENCE**
Cieri,U.R. Determination of reserpine and hydrochlorothiazide in commercial tablets by liquid chromatography with fluorescence and UV absorption detectors in series, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 515–518.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Grind tablet, add 10 mL DMSO, shake vigorously for 5 min, make up to 100 mL with MeOH, mix, filter (paper), discard the first 5 mL filtrate. Dilute 10 mL of the filtrate to 100 mL with MeOH, inject an aliquot.

**HPLC VARIABLES**
**Column:** 75 × 3.9 Novapak silica
**Mobile phase:** MeOH:20 g/L sodium 1-pentanesulfonate in water 100:1
**Flow rate:** 1
**Injection volume:** 20
**Detector:** F ex 280 em 360

**CHROMATOGRAM**
**Retention time:** 5

**OTHER SUBSTANCES**
**Simultaneous:** chlorothiazide (UV 300)

**KEY WORDS**
tablets

**REFERENCE**
Cieri,U.R. Determination of reserpine and chlorothiazide in commercial tablets by liquid chromatography with fluorescence and UV absorbance detectors in series, *J.AOAC Int.*, **1995**, *78*, 1384–1387.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

**HPLC VARIABLES**
**Column:** 100 × 4.6 5 μm Spherisorb SCX
**Mobile phase:** MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid
**Flow rate:** 1
**Injection volume:** 1-10
**Detector:** UV 270

**CHROMATOGRAM**
**Retention time:** 6

**OTHER SUBSTANCES**
**Simultaneous:** cimetidine, clomipramine, halofantrine, haloperidol, minoxidil, verapamil

**REFERENCE**
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Prepare a solution in MeOH, inject a 40 μL aliquot.

**HPLC VARIABLES**
**Column:** 400 × 3 5 μm Nucleosil C18
**Mobile phase:** MeCN:MeOH:acetic acid:water 30:10:0.4:15
**Flow rate:** 1.2
**Injection volume:** 40
**Detector:** F ex 313 em 420

**CHROMATOGRAM**
**Retention time:** 14.7

**OTHER SUBSTANCES**
**Simultaneous:** tilisolol

**REFERENCE**
Yonezawa,K.; Sato,K.; Kobayashi,A. High-performance liquid chromatography of a new β-blocker, 4-[3-(tert.-butylamino)-2-hydroxypropoxy]-N-methylisocarbostyril hydrochloride, in plasma using fluorometric detection, *J.Chromatogr.*, **1985**, *339*, 219–222.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 150 × 4.6 10 μm PRP-1 (Hamilton)
**Mobile phase:** Gradient. MeCN:20 mM ammonium hydroxide from 15:85 to 100:0 over 17 min
**Flow rate:** 1
**Detector:** UV 220

**CHROMATOGRAM**
**Retention time:** 11.5

**OTHER SUBSTANCES**
**Simultaneous:** cocaine, codeine, methadone, thebaine, yohimbine

**REFERENCE**
*Keystone Scientific Catalog, 1993-4*, p. 22.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Lichrosphere cyanopropyl
**Mobile phase:** Carbon dioxide:MeOH:isopropylamine 94:6:0.03
**Column temperature:** 50
**Flow rate:** 3
**Injection volume:** 5
**Detector:** UV 254

### CHROMATOGRAM
**Retention time:** 9.2

### OTHER SUBSTANCES
**Simultaneous:** trifluprom azine, carphenazine, methotrimeprazine, promazine, perphenazine, chlorprothixene, deserpidine, thiothixene
**Also analyzed:** acetophenazine, ethopropazine, promethazine, propiomazine

### KEY WORDS
SFC; pressure 200 bar

### REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

### SAMPLE
**Matrix:** solutions

### HPLC VARIABLES
**Column:** 250 × 4.6 Zorbax RX
**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
**Column temperature:** 30
**Flow rate:** 2
**Detector:** UV 210

### OTHER SUBSTANCES
**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine,

quinine, ranitidine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

**REFERENCE**

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

**SAMPLE**

**Matrix:** solutions

**HPLC VARIABLES**

**Column:** 150 × 4.6 Develosil ODS-5

**Mobile phase:** MeOH:water 70:30

**Flow rate:** 1

**Detector:** MS, JEOL JMS-SX102A reversed geometry (BE), accelerating voltage +5 kV, air pressure chemical ionization APCI, nebulizer 300°, ion source chamber 400°, discharge electrode, skimmer 1 aperture 300 μm, skimmer 2 aperture 400 μm, no nebulizer gas, m/z 608

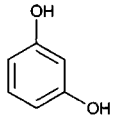
**CHROMATOGRAM**

**Limit of quantitation:** 10 pg

**REFERENCE**

Nojima,K.; Fujimaki,S.; Hertsens,R.C.; Morita,T. Application of liquid chromatography-atmospheric pressure chemical ionization mass spectrometry to a sector mass spectrometer, *J.Chromatogr.A*, **1995**, *712*, 17–19.

# Resorcinol




**Molecular formula:** $C_6H_6O_2$

**Molecular weight:** 110.11

**CAS Registry No.:** 108-46-3

**Merck Index:** 8323

**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

**HPLC VARIABLES**

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 200

**CHROMATOGRAM**
**Retention time:** 8.027

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Inject an aliquot of a solution in MeOH:50 mM pH 3.0 triethylamine phosphate 40:60.

**HPLC VARIABLES**
**Column:** 150 × 3.2 5 μm Hypersil ODS
**Mobile phase:** THF:50 mM pH 3.0 triethylamine phosphate 12:88
**Flow rate:** 0.6
**Injection volume:** 20
**Detector:** UV 275 following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID crocheted PTFE coil irradiated with an 8 W low-pressure mercury lamp at 254 nm to the detector.

**CHROMATOGRAM**
**Retention time:** 9

**OTHER SUBSTANCES**
**Simultaneous:** acetaminophen (post-column irradiation gives little increase in peak height), aspirin, caffeine (post-column irradiation gives little increase in peak height), propyphenazone (post-column irradiation gives a decrease in peak height)

**KEY WORDS**
post-column photochemical derivatization

**REFERENCE**
Di Pietra,A.M.; Gatti,R.; Andrisano,V.; Cavrini,V. Application of high-performance liquid chromatography with diode-array detection and on-line post-column photochemical derivatization to the determination of analgesics, *J.Chromatogr.A*, **1996**, *729*, 355–361.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Aqueous food simulants. Pipette 1.0 mL 200 mg/L IS in MeOH into a 25 mL volumetric flask and dilute to the mark with the food simulant obtained from migration experiment, shake. Repeat the procedure to obtain a duplicate sample, filter a portion through a 0.2 μm membrane filter, inject a 20 μL aliquot. Olive oil simulants. Weigh 25 g olive oil food simulant obtained from migration experiment into a beaker, pour oil into a separating funnel, allow beaker to drain for 30 s. Rinse it with 25 mL hexane and add washes to separating funnel. Add 1.0 mL 200 mg/L IS in MeOH into funnel and mix. Add 10 mL water, shake vigorously by hand for 30 s, allow to stand for 5 min. Collect aqueous phase and reextract oil with a 10 mL water. Combine aqueous extracts, make up to25 mL with water, filter the extracts through a small cotton plug to remove any entrained oil. Repeat the procedure to obtain a duplicate sample. Inject a 20 μL aliquot. (Aqueous food simulants were: distilled water, 3% acetic acid in water; EtOH:water 15:85.)

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Hypersil ODS
**Mobile phase:** MeCN:buffer 15:85 (Prepare mobile phase as follows. Dissolve 7.5 g sodium dihydrogen orthophosphate in 800 mL water, add 150 mL MeCN and adjust to pH 3.6 with glacial acetic acid. Make up to 1000 mL with water.)
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 280

**CHROMATOGRAM**
**Retention time:** 6.5
**Internal standard:** 2-methyl-1,3-dihydroxybenzene (7.4)
**Limit of detection:** 100 ng/g

**OTHER SUBSTANCES**
**Extracted:** hydroquinone, pyrocatechol

**KEY WORDS**
aqueous food simulants; olive oil simulants

**REFERENCE**
Philo,M.R.; Jickells,S.M.; Castle,L. Testing for compliance with migration limits: Determination of 1,2-, 1,3-, and 1,4-dihydroxybenzenes in food-simulating solvents by liquid chromatography, *J.AOAC Int.*, **1996**, *79*, 746–750.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 250 × 4.6 10 μm LiChrosorb RP 18
**Mobile phase:** MeOH:10 mM pH 5.5 potassium phosphate buffer 3.5:96.5
**Flow rate:** 2-3
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 10

**OTHER SUBSTANCES**
**Simultaneous:** catechol, hydroquinone (quinol), phenol, phenyl glucuronide, phenyl glucoside, phenyl galactopyranoside, phenyl sulfate

**REFERENCE**
Beyer,J.; Frank,G. Hydroxylation and conjugation of phenol by the frog *Rana temporaria*, *Xenobiotica*, **1985**, *15*, 277–280.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Inject an aliquot of an aqueous solution.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Microsorb C8
**Mobile phase:** Gradient. MeOH:1% acetic acid in water from 0:100 to 75:25 over 25 min, to 100:0 over 0.5 min maintain at 100:0 for 5.5 min, return to initial conditions over 1 min, re-equilibrate for 7 min.
**Column temperature:** 35
**Flow rate:** 1.2
**Injection volume:** 20
**Detector:** UV 280

**CHROMATOGRAM**
**Retention time:** 10

**OTHER SUBSTANCES**
**Simultaneous:** phthalic acid, 2-(2',4'-dihydroxybenzoyl)benzoic acid

**REFERENCE**
Calvey,R.J.; Goldberg,A.L. Liquid chromatographic determination of intermediates in D&C Yellow No. 7 and D&C Yellow No. 8, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 471–473.

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**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 250 × 4.6 Spheri 10 RP-18
**Mobile phase:** MeOH:water 36:64 containing 20 mM $KH_2PO_4$
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 230

**CHROMATOGRAM**
**Retention time:** 4.25

**OTHER SUBSTANCES**
**Simultaneous:** phthalic acid

**REFERENCE**
Lancaster,F.E.; Lawrence,J.F. High-performance liquid chromatographic determination of subsidiary dyes, intermediates and side reaction products in erythrosine, *J.Chromatogr.*, **1987**, *388*, 248–252.

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**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Inject a 20 μL aliquot of a solution in 1% acetic acid.

**HPLC VARIABLES**
**Guard column:** 30 × 4.6 Spheri-5 RP-18
**Column:** 250 × 4.6 5 μm Ultrasphere-ODS C18
**Mobile phase:** Gradient. A was MeCN:acetic acid 99:1. B was 1% acetic acid in water. A:B from 0:100 to 10:90 over 10 min, to 20:80 over 25 min, wash with A for 6 min, re-equilibrate for 14 min.
**Flow rate:** 2
**Injection volume:** 20
**Detector:** F ex 284 em 313

**CHROMATOGRAM**
**Retention time:** 6

**OTHER SUBSTANCES**
**Simultaneous:** catechol (F ex 280 em 325), hydroquinone (F ex 304 em 338), phenol (ex 274 em 298)

**REFERENCE**
Risner,C.H.; Cash,S.L. A high-performance liquid chromatographic determination of major phenolic compounds in tobacco smoke, *J.Chromatogr.Sci.*, **1990**, *28*, 239–244.

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**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 250 × 4.6 Zorbax RX
**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
**Column temperature:** 30
**Flow rate:** 2
**Detector:** UV 210

## OTHER SUBSTANCES

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

## REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

## SAMPLE

**Matrix:** solutions
**Sample preparation:** Prepare a 1-10 μg/mL solution in water, inject an aliquot.

## HPLC VARIABLES

**Column:** 250 × 4.6 5 μm Hypersil SCX/C18
**Mobile phase:** MeCN:25 mM pH 3 $Na_2HPO_4$ 50:50
**Injection volume:** 20
**Detector:** UV 254

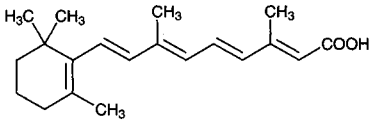
**CHROMATOGRAM**
**Retention time:** k' 0.38

**OTHER SUBSTANCES**
**Also analyzed:** amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, salicylic acid, secobarbital, terbutaline, xylazine

**KEY WORDS**
effect of mobile phase pH on capacity factor is discussed

**REFERENCE**
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

# Retinoic acid




**Molecular formula:** $C_{20}H_{28}O_2$
**Molecular weight:** 300.44
**CAS Registry No.:** 302-79-4 (tretinoin (all-trans)), 4759-48-2 (isotretinoin (13-cis)), 5300-03-8 (alitretinoin (9-cis))
**Merck Index:** 8333
**Lednicer No.:** 3 12 (isotretinoin)

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Evaporate 25-100 μL IS in MeOH to near dryness under a stream of nitrogen. Add 0.2-1 mL plasma and 100 μL buffer, extract with 2 mL diethyl ether:ethyl acetate 50:50 for 5 min. Centrifuge at 2000 g for 10 min at 4°, evaporate the organic phase to dryness. Dissolve the residue in 30-100 μL MeOH, inject an aliquot. (Solution was prepared in yellow amber glass and all handling was performed in a room with dim yellow light! Buffer was 25 mM $KH_2PO_4$ containing 40 mM $Na_2HPO_4$, pH 7.)

**HPLC VARIABLES**
**Column:** 250 × 4.6 4 μm Nova-Pak C18
**Mobile phase:** Gradient. A was MeCN:MeOH:THF 33.25:61.75:5. B was 2% acetic acid. A:B from 75:25 to 88:12 over 11 min, maintain at 88:12 for 19 min, return to initial condition at 30 min, equilibrate for 10 min.
**Flow rate:** 1
**Injection volume:** 25
**Detector:** UV 350

**CHROMATOGRAM**
**Retention time:** 24.9 (isotretinoin), 26.7 (tretinoin), 26.2 (alitretinoin (9-cis))
**Internal standard:** acitretin (21), 13-cis-acitretin (19.8)
**Limit of quantitation:** 2 ng/mL

**OTHER SUBSTANCES**
**Extracted:** metabolites
**Noninterfering:** acetaminophen, acyclovir, alprazolam, amikacin, amitriptyline, amphotericin B, aspirin, atenolol, bromazepam, caffeine, carbamazepine, ceftriaxone, chlorpromazine, cimetidine, clonazepam, dextromethorphan, diazepam, erythromycin, flunitrazepam, haloperidol, ketoconazole, lorazepam, meprobamate, metronidazole, methylprednisolone, miconazole, midazolam, nifedipine, nitrazepam, netilmicin, nordiazepam, nystatin, oxazepam, phenytoin, prednisolone, prednisone, sulconazole, theophylline, thiopental, zidovudine

**KEY WORDS**
plasma; rabbit; rat